SUPPLEMENTAL MATERIALS

Establishment of a method to rapidly assay bacterial persister metabolism

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Running title: Persister metabolism

Table S1. Dead, VBNC and persister levels in bacterial cultures before and after the antibiotic treatment.

WT	Before antibiotic treatment	After antibiotic treatment
Normal cells (%)	88.42 ± 3.40	-
Dead cells (%)	6.84 ± 0.93	85.26 ± 5.89
VBNCs (%)	4.68 ± 2.55	14.68 ± 5.89
Persisters (%)	0.06 ± 0.00	0.06 ± 0.00
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Normal cells (%)	87.66 ± 0.90	-
Dead cells (%)	5.56 ± 1.15	91.74 ± 3.02
VBNCs (%)	5.83 ± 0.89	7.31± 2.88
Persisters (%)	0.95 ± 0.21	0.95 ± 0.21

Table S2. Cell growth in phenotype microarray (ΔOD_{600} after 6 hours of incubation).

Negative control 0.00±0.00	L-Arabinose 0.74±0.05	N-Acetyl-D-Glucosamine 1.13±0.07	D-Saccharic Acid 0.02±0.02	Succinic Acid 0.47±0.11	D-Galactose 0.17±0.02	L-Aspartic Acid 0.31±0.06	L-Proline 0.00±0.00	D-Alanine 0.09±0.04	D-Trehalose 0.22±0.03	D-Mannose 0.28±0.04	Dulcitol 0.13±0.13
D-Serine 0.24±0.03	D-Sorbitol 0.35±0.03	Glycerol 0.53±0.04	L-Fucose 0.38±0.03	D-Glucoronic Acid 0.60±0.04	D-Gluconic Acid 0.55±0.06	D,L-α-Glycerol P. 0.26±0.06	D-Xylose 0.45±0.04	L-Lactic Acid 0.30±0.05	Formic Acid 0.00±0.00	D-Mannitol 0.72±0.07	L-Glutamic Acid 0.00±0.00
D-Glucose-6-Phosphate 0.60±0.03	D-Galactonic Acid-γ-Lactone 0.33±0.03	D,L-Malic Acid 0.35±0.05	D-Ribose 0.07±0.01	Tween 20 0.26±0.05	L-Rhamnose 0.13±0.03	Fructose 0.75±0.07	Acetic Acid 0.08±0.03	α-D-Glucose 1.47±0.12	Maltose 0.49±0.02	D-Melibiose 0.29±0.06	Thymidine 0.32±0.05
L-Asparagine 0.24±0.03	D-Aspartic acid 0.00±0.00	D-Glucosaminic Acid 0.00±0.00	1,2-Propanediol 0.00±0.00	Tween 40 0.25±0.04	α-Keto-Glutaric Acid 0.26±0.07	α-Keto-Butyric Acid 0.18±0.03	α-Methyl-D-Galactoside 0.39±0.05	α- D-Lactose 0.77±0.04	Lactulose 0.01±0.01	Sucrose 0.00±0.00	Uridine 0.24±0.02
L-Glutamine 0.01±0.01	m-Tartaric Acid 0.05±0.03	D-Glucose-1-Phosphate 0.66±0.05	D-Fructose-6-Phosphate 0.62±0.07	Tween 80 0.23±0.03	α-Hydroxy Glutaric A.-γ-Lactone 0.02±0.01	α-Hydroxy Butyric Acid 0.20±0.05	β-Methyl-D-Glucoside 0.13±0.03	Adonitol 0.01±0.01	Maltotriose 1.00±0.07	2-Deoxy Adenosine 0.37±0.03	Adenosine 0.27±0.02
Glycyl-L-Aspartic Acid 0.28±0.03	Citric Acid 0.00±0.00	m-Inositol 0.00±0.00	D-Threonine 0.00±0.00	Fumaric Acid 0.34±0.07	Bromo Succinic Acid 0.21±0.04	Propionic Acid 0.11±0.04	Mucic Acid 0.36±0.03	Glycolic Acid 0.04±0.04	Glyoxylic Acid 0.31±0.27	D-Cellobiose 0.01±0.01	Inosine 0.31±0.06
Glycyl-L-Glutamic Acid 0.19±0.05	Tricarballylic Acid 0.01±0.01	L-Serine 0.39±0.05	L-Threonine 0.06±0.03	L-Alanine 0.30±0.05	L-Alanyl-Glycine 0.35±0.07	Acetoacetic acid 0.03±0.02	N-Acetyl-β-D-Mannosamine 0.11±0.04	Mono Methyl Succinate 0.03±0.03	Methyl Pyruvate 0.02±0.02	D-Malic Acid 0.30±0.09	L-Malic Acid 0.33±0.09
Glycyl-L-Proline 0.25±0.01	p-Hydroxy Phenyl Acetic Acid 0.02±0.02	m-Hydroxy Phenyl Acetic Acid 0.01±0.01	Tyramine 0.00±0.00	D-Psicose 0.00±0.00	L-Lyxose 0.00±0.00	Glucuronamide 0.02±0.02	Pyruvic acid 0.29±0.03	L-Galactonic Acid-γ-Lactone 0.31±0.06	D-Galacturonic Acid 0.73±0.08	Phenylethyl amine 0.00±0.00	2-Aminoethanol 0.00±0.00

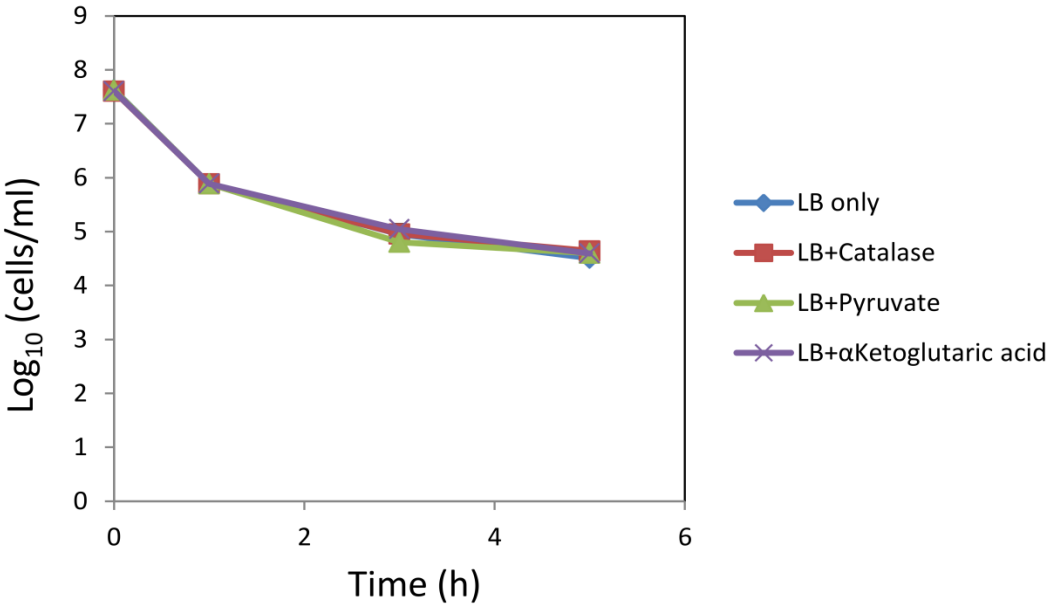

Figure S1. Resuscitation of VBNCs. Exponential phase cells treated with ampicillin for 5 hours were plated on LB, LB + pyruvate (0.1%), LB + α-ketoglutarate (0.1%) and LB + catalase (2000U/ plate) agar plates (replicated 3 times). Error bars indicate the standard error of the mean.

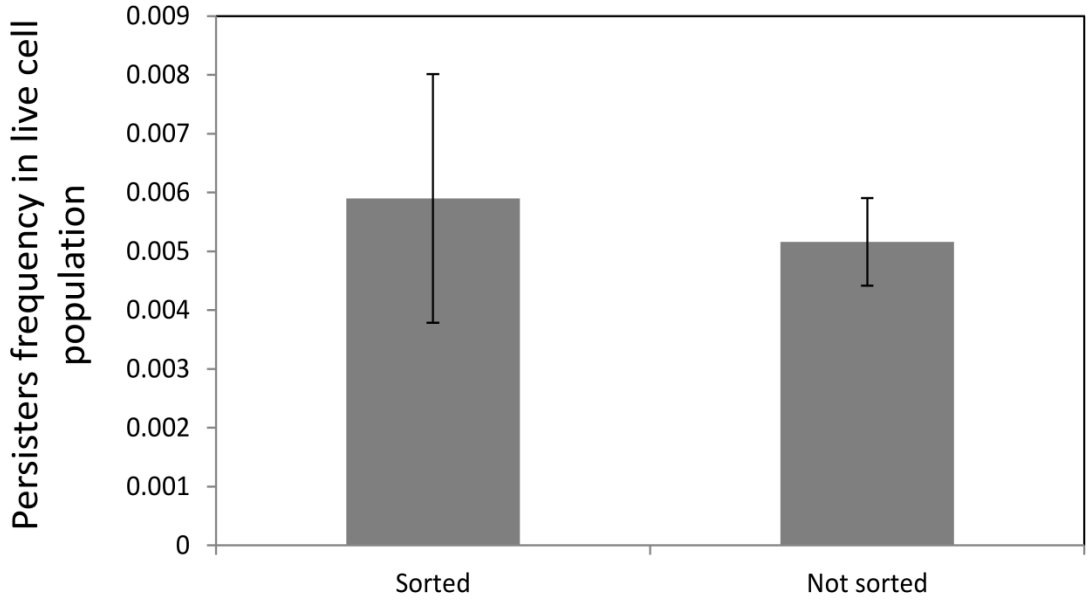

Figure S2. Persister frequency in live cell population after ampicillin treatment. Approximately 10,000 mCherry positive cells (live) after 5 hour ampicillin treatment were sorted and plated on LB agar to count CFUs (persisters). Ampicillin-treated cultures were also plated without sorting, and the number of live cells (mCherry positive cells) was enumerated by flow cytometry with fluorescent counting particles. Error bars indicate the standard error of the mean.

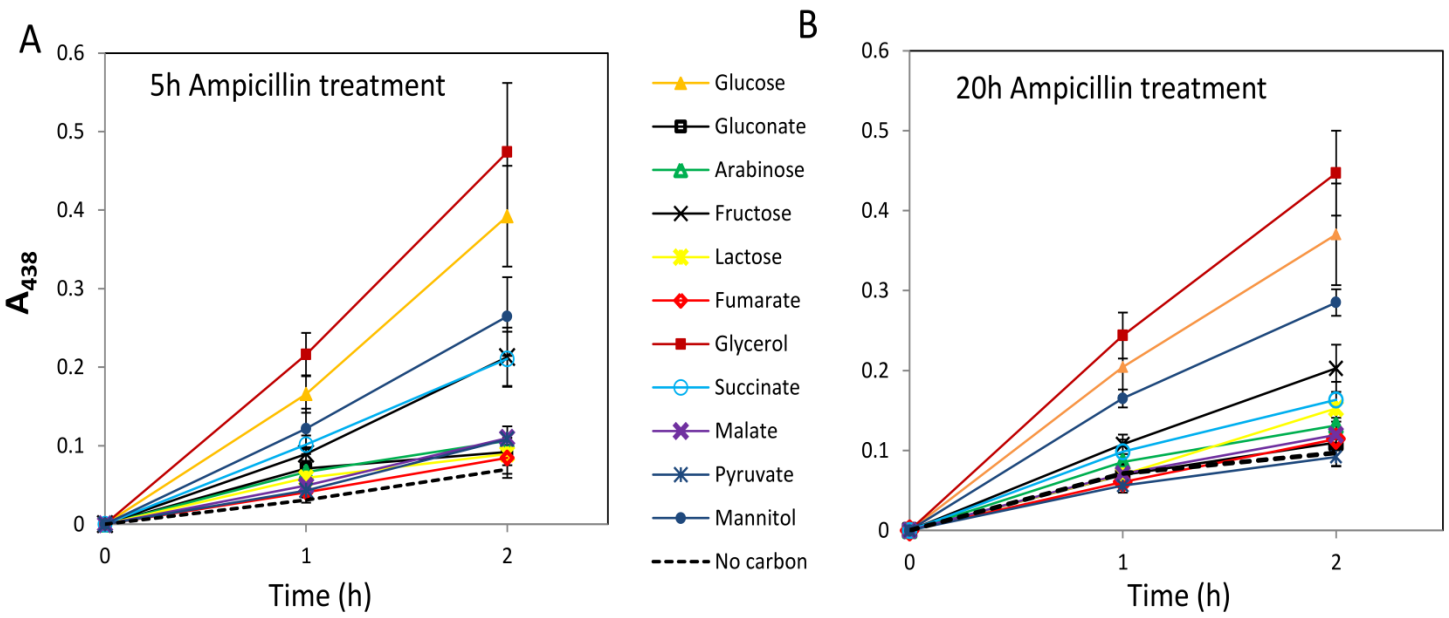

Figure S3. Metabolic activity measurement of ampicillin-treated cultures. Live cells (VBNCs and persisters) after 5 hour (A) or 20 hour (B) ampicillin treatment were incubated in M9 minimal media with various carbon sources, WST-1 and electron mediator by using microplates, and absorbance (A_{438}) was measured at indicated time points. Error bars indicate the standard error of the mean.

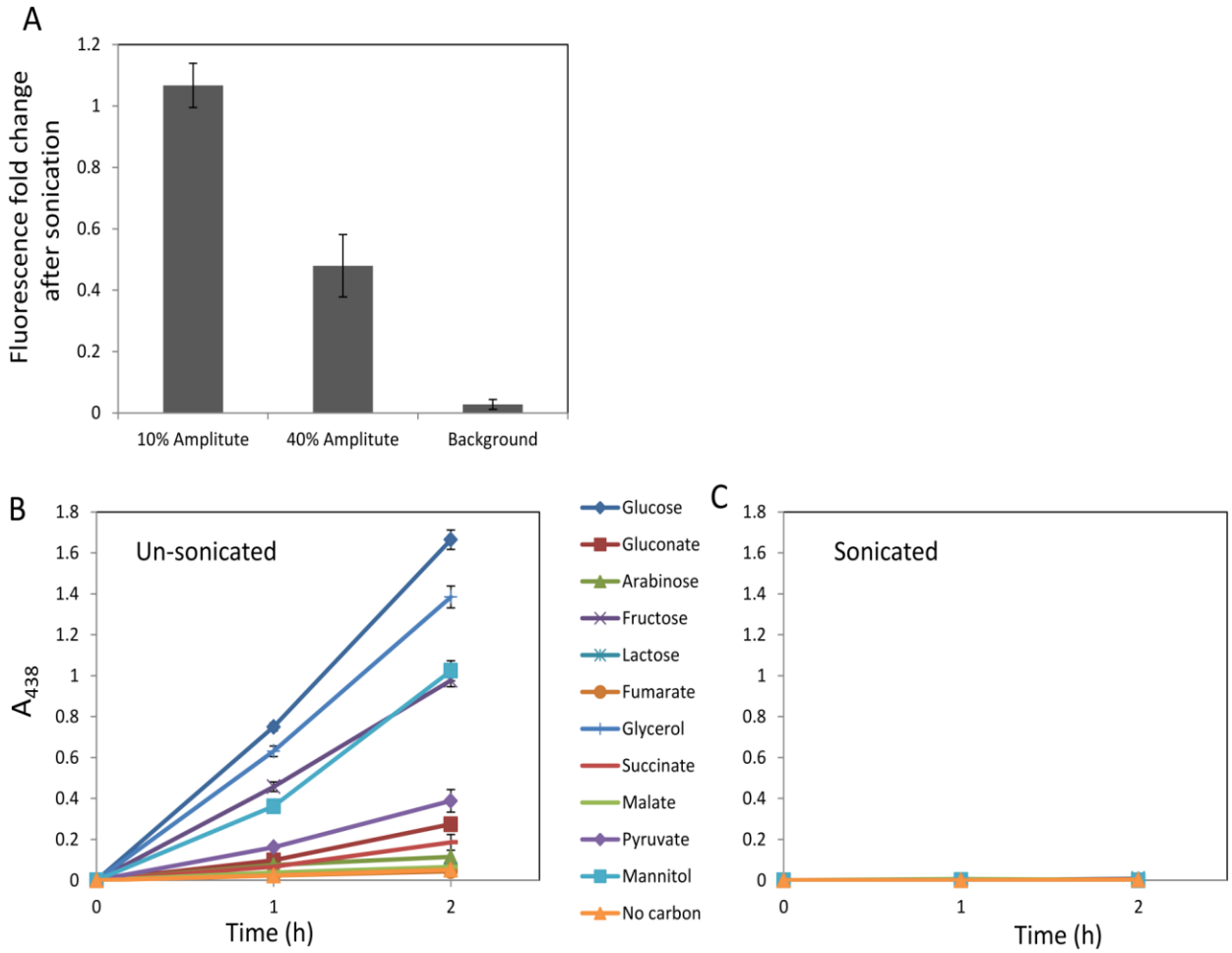

Figure S4. Comparison of metabolic activities of lysed (sonicated) and exponential phase cells. Exponential phase MO001 cultures (2mL) were washed and diluted in M9 salt solution and sonicated at the specified amplitude for 30 minutes on ice, fluorescence was measured before and after sonication using a plate reader (560 nm excitation, 610nm emission) (A). Data indicated that 10% amplitude failed to denature mCherry, but mCherry could serve as an indicator of denaturation, since 40% amplitude significantly reduced fluorescence. Background was fluorescence of sonicated wild-type cells (non-mCherry expressing) during exponential phase. Fluorescence intensities measured after sonication were normalized to fluorescence intensities of unsonicated MO001 cultures. Both sonicated (10% amplitude) and un-sonicated cells chilled on ice were incubated in M9 media with various carbon sources and absorbance measurements were taken at indicated time points (B and C). Error bars indicate the standard error of the mean.

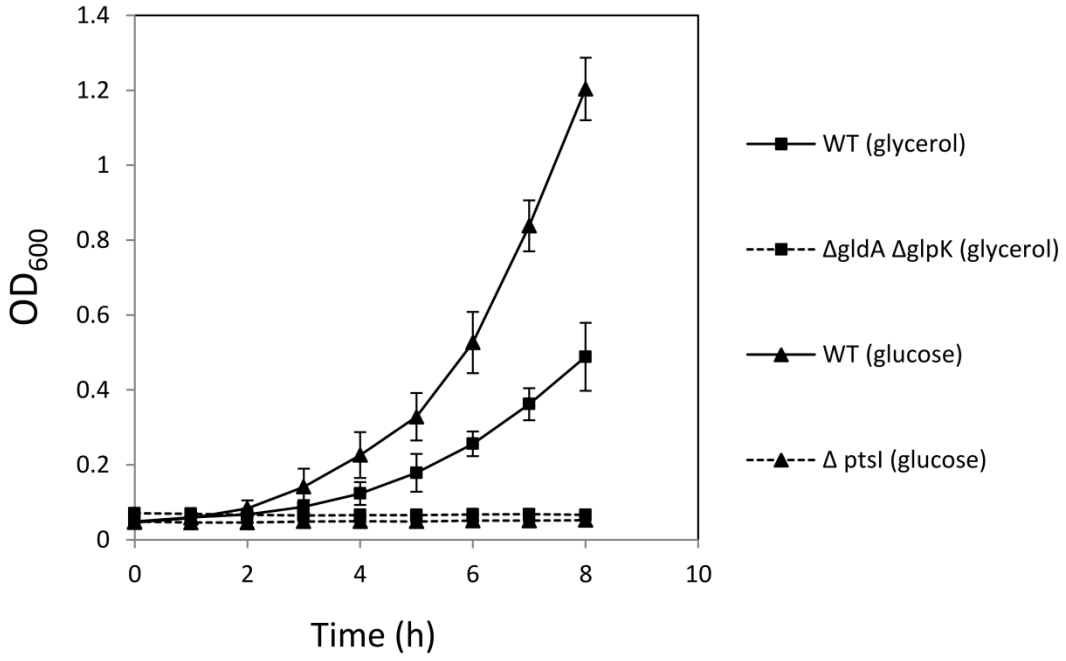

Figure S5. Growth curves of *ΔgldA ΔglpK* and *ΔptsI* in minimal media. Overnight cells in LB were diluted 100-fold in M9 minimal media with indicated carbon sources (60mM carbon) in 250mL flask, and incubated at 37°C and 250rpm. OD_{600} measurements were taken at indicated time points. Error bars indicate the standard error of the mean.

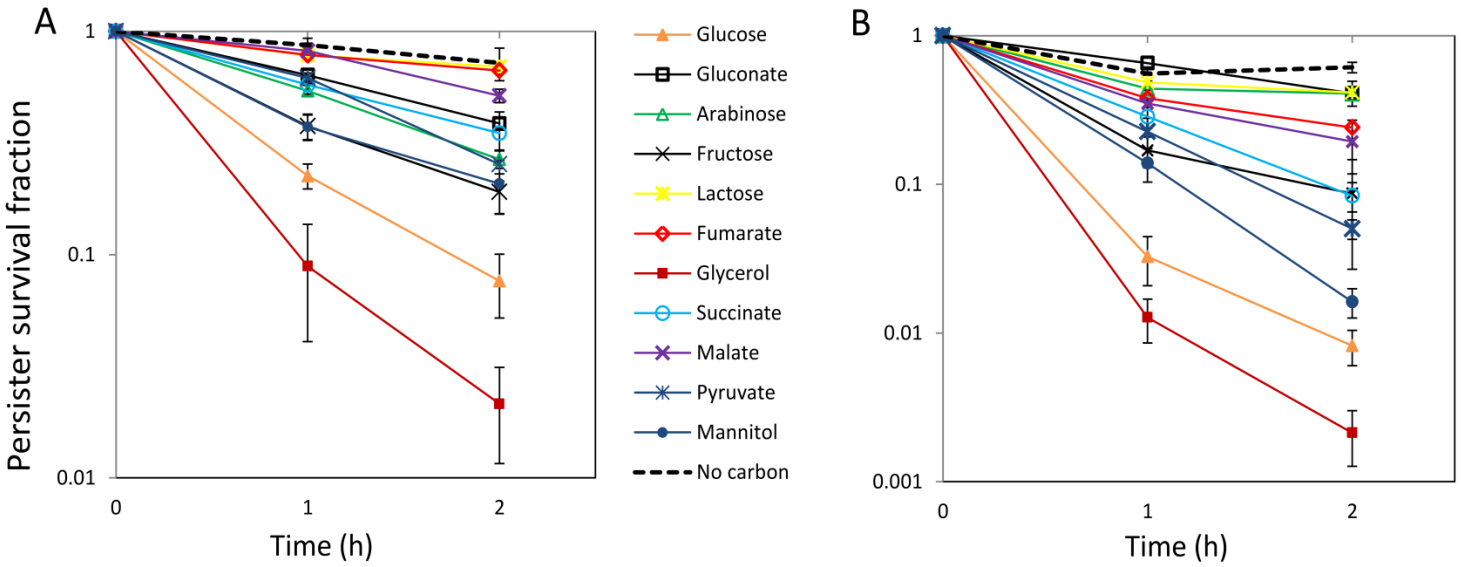

Figure S6. AG potentiation assay for exponential-phase persisters. After exponential-phase cells were treated with ampicillin (A) or ofloxacin (B) for 5 hours, cells were incubated with kanamaycin (KAN) (25µg/mL) in M9 minimal media with different carbon sources using 96-well plates. Changes in CFUs were monitored during the 2 hour assay. Error bars indicate the standard error of the mean.

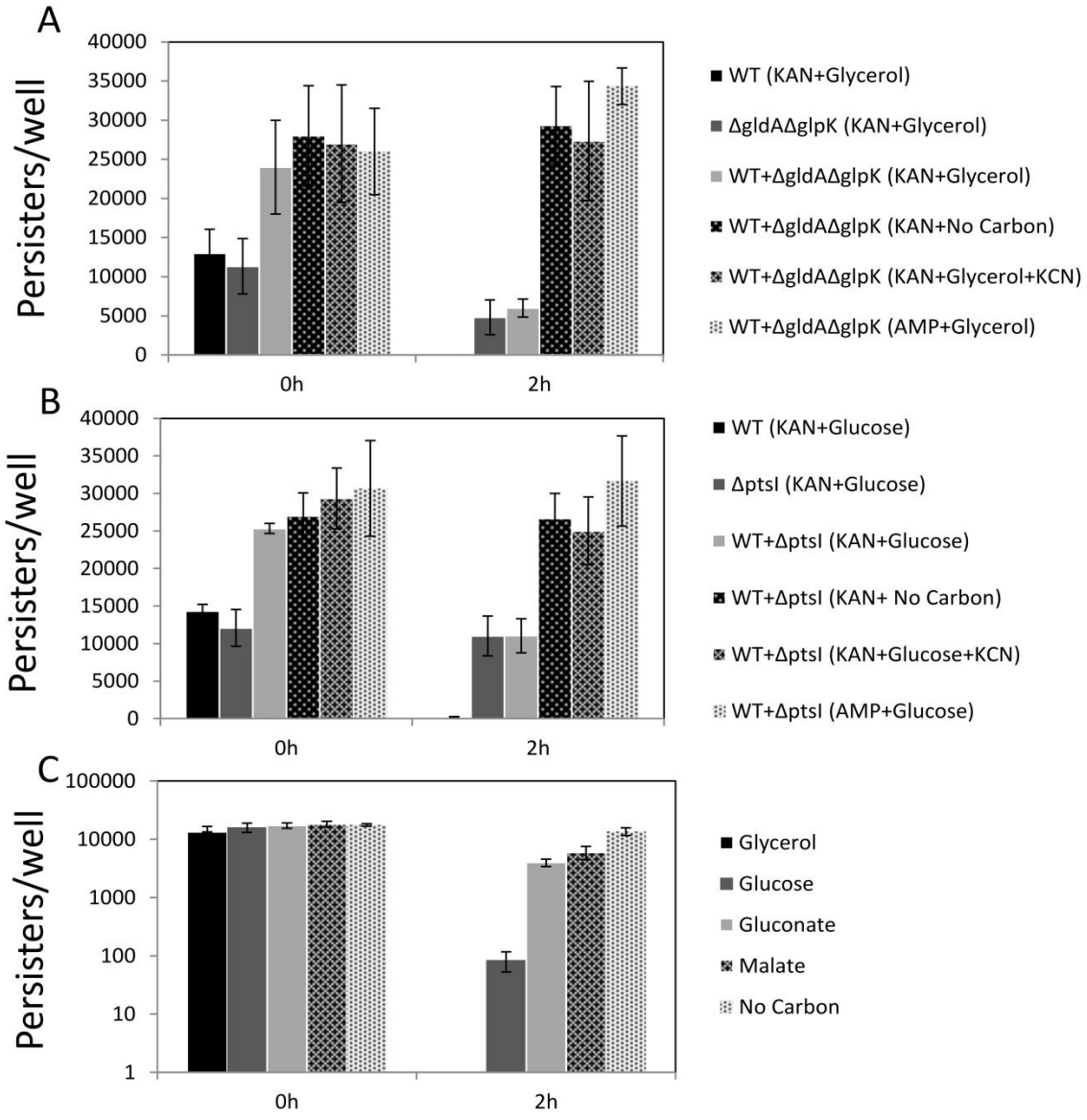

Figure S7. Competition assay for exponential-phase cells treated with ofloxacin. After 5 hour treatment of exponential phase cells with OFX, wild-type only, *ΔgldAΔglpK* only and mixed cell cultures (approximately 50/50 proportion of persisters) at low cell densities were treated for 2 hours with KAN and glycerol (A). The same experiments were repeated for wild-type and *ΔptsI* cultures (B). When KCN was used with KAN, AG potentiation was eliminated. Without a carbon source, all cells survived in the presence of KAN. AG potentiation of various carbon sources was tested in low cell density assay (C). Note that, in low cell density assays, no detectable CFUs were counted after 2 hour of treatment with KAN and glycerol (A and C). Error bars indicate the standard error of the mean.

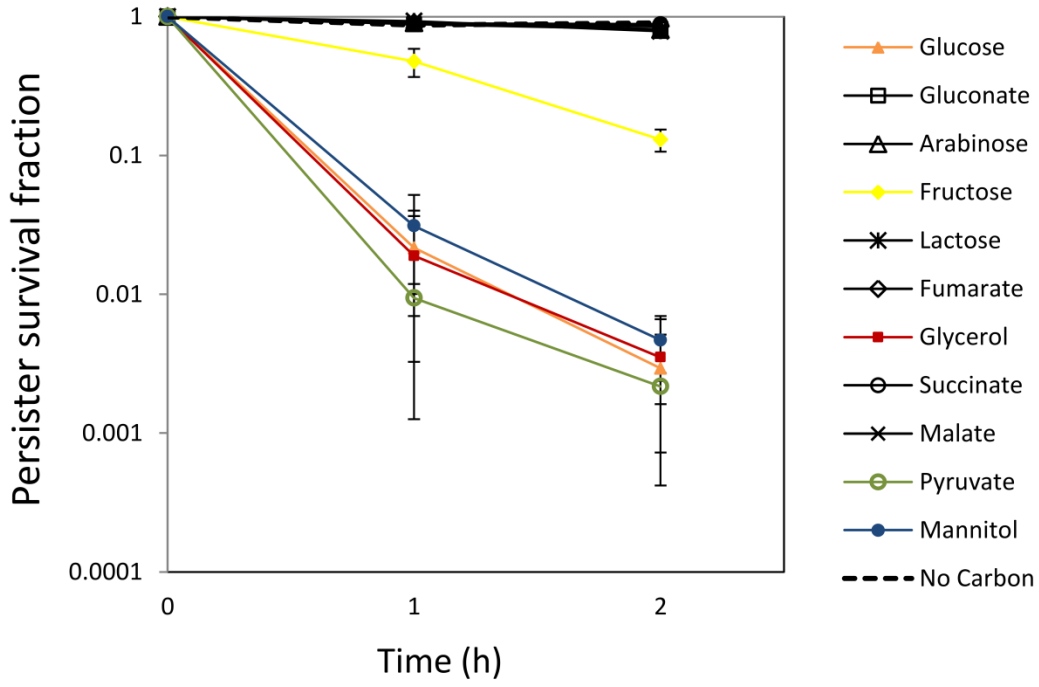

Figure S8. AG potentiation assay for stationary-phase persisters. After stationary-phase cells were treated with OFX for 5 hours, cells were incubated with kanamycin (KAN) (25µg/mL) in M9 minimal media with different carbon sources using 96-well microplates. Survival fraction was monitored by CFU. Error bars indicate the standard error of the mean.

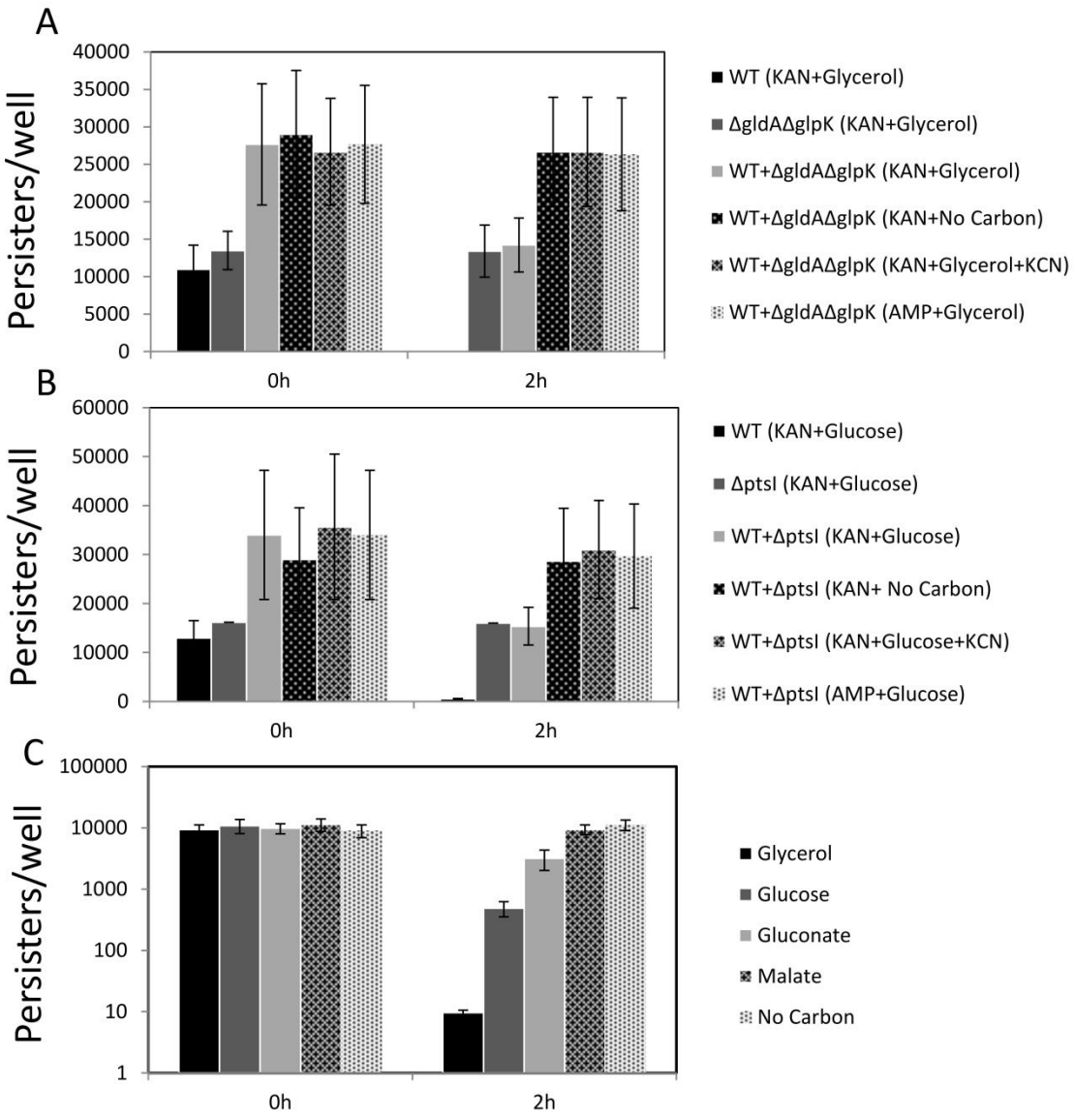

Figure S9. Competition assay for stationary-phase cells treated with ofloxacin. After 5 hour treatment of stationary-phase cells with OFX, wild-type only, *ΔgldAΔglpK* only and mixed cell cultures (50/50 proportion of persisters) at low cell densities were treated with KAN and glycerol for 2 hours (A). The same experiments were repeated for wild-type and *ΔptsI* cultures (B). AG potentiation of various carbon sources was tested in low cell density assay (C). Error bars indicate the standard error of the mean.

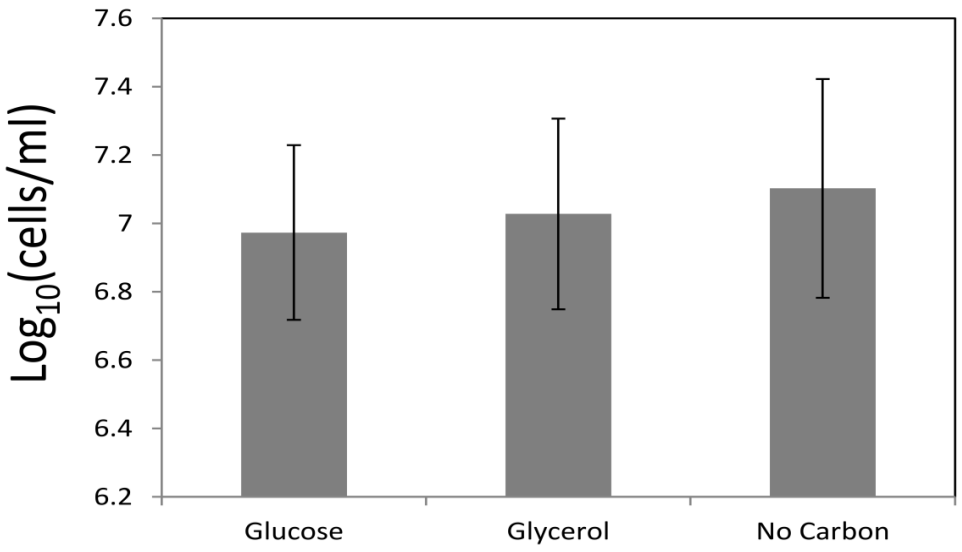

Figure S10. Enumeration of live cells during AG assay. Ampicillin-treated exponential phase cells were treated with KAN and glucose or glycerol. The cells were stained with SYTO9 and PI after 2 hour of AG treatment, and analyzed by flow cytometry to enumerate live cells. As a control, ampicillin-treated cells were not exposed to a carbon source during the AG treatment. Error bars indicate the standard error of the mean.

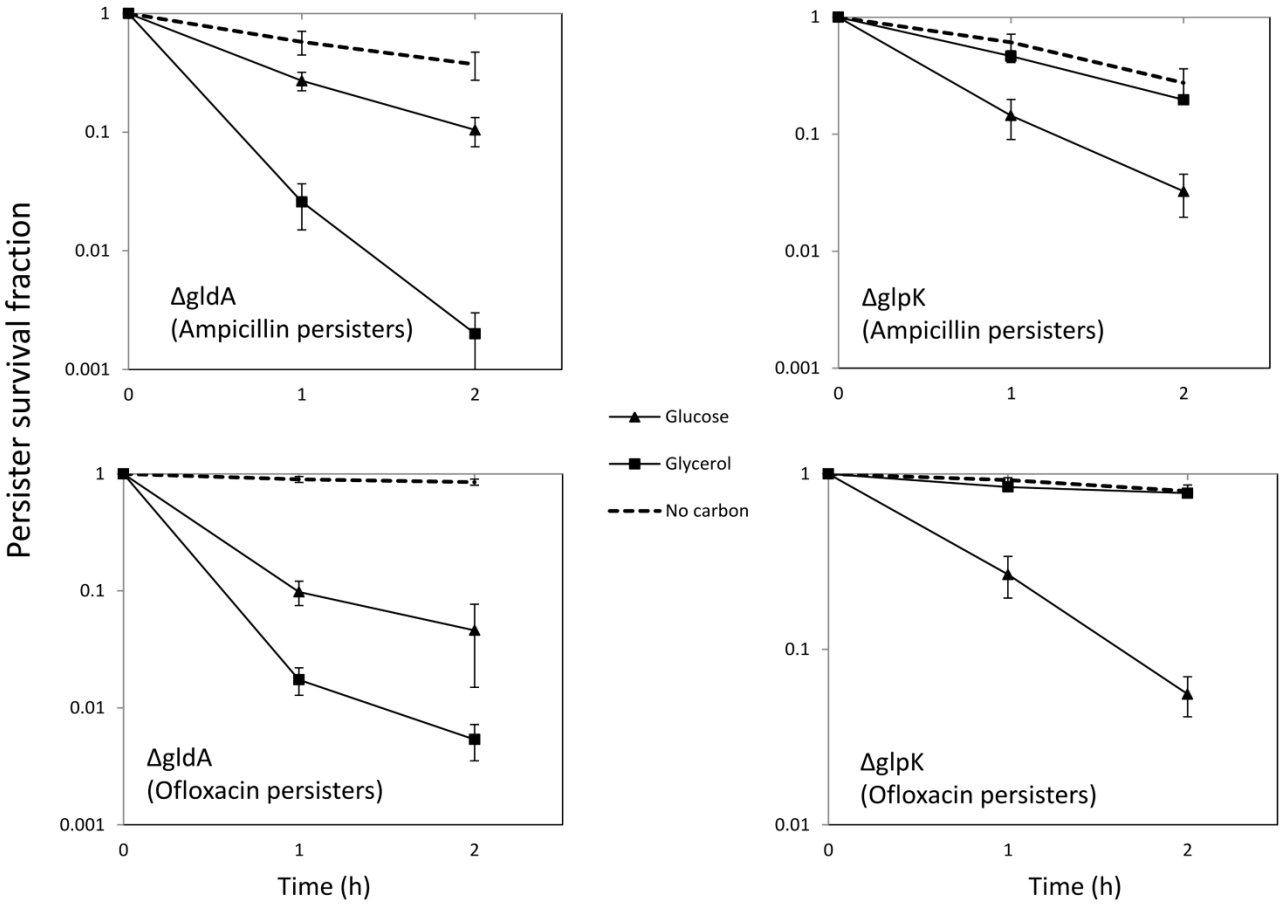

Figure S11. Identification of active glycerol degradation pathway in persisters. Ampicillin and ofloxacin persisters from *ΔgldA* and *ΔglpK* cell types at exponential phase stage were treated with kanamycin in M9 minimal media with indicated carbon sources, and the persister survival fractions were monitored for 2 hours. Error bars indicate the standard error of the mean.